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# Soil Organic Nitrogen Indirectly Enhances Pepper-Residue-Mediated Soil Disease Suppression through Manipulation of Soil Microbiome

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**Abstract:** Banana *Fusarium* wilt-suppressive soils are effective against pathogen invasion, yet soil physicochemical factors responsible for conducive or suppressive behavior have not been reported. Here, we investigated the changes in banana biomass, disease incidence (DI), soil culturable microbes and physicochemical properties by incorporating pepper and banana residues into conducive and suppressive soils. Before the incorporation of any residues, the suppressive soil significantly increased banana biomass and decreased DI compared to the conducive soil. The biomass of the suppressive soil was significantly higher than that of the conducive soil after the incorporation of either pepper or banana residues. Compared with the control (CK), the incorporation of pepper residues to both soils significantly reduced DI, while banana residues had the opposite effect. Additionally, both conducive and suppressive soils supplemented with pepper residues significantly reduced the amounts of culturable *Fusarium oxysporum* and increased the amounts of beneficial *Pseudomonas* and *Bacillus*. The pepper residue extracts significantly inhibited the growth of *F. oxysporum* mycelium. Soil alkali-hydrolyzable nitrogen (AN) responded most strongly to residue application to suppressive soil. The AN factor was significantly and positively correlated with banana biomass; however, there was no direct and significant negative correlation with DI. Further analysis of the results showed that elevated AN content could stimulate the amounts of culturable *Bacillus* in the soil, and *Bacillus* antagonized the proliferation of pathogen and thus indirectly and effectively reduced banana DI. In conclusion, soil AN content can indirectly improve the disease suppression ability of pepper-residue-mediated suppressive soil by manipulating the soil microbiome.

**Keywords:** conducive soil; suppressive soil; plant residue; *Fusarium* wilt disease; culturable microbes; alkali-hydrolyzable nitrogen



**Citation:** Hong, S.; Jv, H.; Yuan, X.; Geng, J.; Wang, B.; Zhao, Y.; Wang, Q.; Li, R.; Jia, Z.; Ruan, Y. Soil Organic Nitrogen Indirectly Enhances Pepper-Residue-Mediated Soil Disease Suppression through Manipulation of Soil Microbiome. *Agronomy* **2022**, *12*, 2077. https://doi.org/10.3390/agronomy12092077

Academic Editor: Joji Muramoto

Received: 19 June 2022
Accepted: 25 August 2022
Published: 31 August 2022



## 1. Introduction

The soil-borne fungal pathogen *Fusarium oxysporum* has a global distribution and can infect a variety of host plants, leading to *Fusarium* wilt [1]. Susceptible crops that have been reported worldwide include cotton, tomato, cucumber, muskmelon and banana [2]. Banana is an important cash and food crop in tropical regions. Banana *Fusarium* wilt-suppressive soils are usually defined as soils where the pathogen cannot colonize or where

the pathogen can colonize but causes little damage to banana plants and where disease incidence (DI) decreases or is even absent with successive planting of banana [3–5]. In contrast, conducive soils correspond to soils that are susceptible to disease. It has been reported that abiotic factors such as pH and nutrient content [6] and biotic factors such as *Pseudomonas* and *Bacillus* [7] are associated with the suppressive or conducive nature of soil; in other words, these factors are important indicators of the suppressive ability of a soil. In agricultural production, conducive soils can be induced to form a suppressive soil by successive cultivation of susceptible crops [8], biofertilizer application [9], crop rotation practices [10] and incorporation of plant residues; among these techniques, incorporating plant residues is a relatively important and effective but time-consuming practice [11].

The application of residues in fields was found to effectively reduce the crop disease incidence during the season of application. For example, the application of residues such as pineapple, dasheen and komatsuna could significantly elevate the crop disease suppression effects within the season by improving soil physicochemical properties, promoting nutrient recycling, regulating soil microbial community composition, restoring soil microecological balance and inhibiting the explosive growth of single harmful microorganisms [12–15]. Moreover, plant residue decay induces colonization by residual host-specific microbes of different morphological, functional and microbial activities in suppressive soils to resist pathogen invasion [11,16]. Therefore, the study of the processes by which plant residues affect microbial disease suppression is equally important for agricultural production.

Naturally formed suppressive soils protect plants from pathogens [17–19]. In addition, it has been found that suppressive and conducive soils often differ significantly in their biophysicochemical properties in addition to their microbial community composition [20,21]. The soil physicochemical environment significantly affects the antimicrobial stability of beneficial functional microbes [22]. For example, a high phosphorus content in soil can reduce the relative abundance of beneficial microbes and induce an increase in the abundance of pathogens leading to peanut *Fusarium* wilt [23]. The basis of our previous study showed that *Pseudomonas* and the soil physicochemical properties such as available phosphorus (AP) were significantly correlated with disease suppression in banana *Fusarium* wilt naturally suppressive soils [5]. We then confirmed that the incorporation of pineapple residues in the conducive soil alleviates the pathogen pressure and thus reduces the DI of banana, whereas the addition of banana residues exacerbates banana *Fusarium* wilt disease [11]. We also confirmed that crop rotation of pepper is an effective agricultural management strategy to reduce banana DI in the field settings [24]. In addition, local farmers were returning pepper residues to the field after implementing pepper–banana rotation strategies for the development of suppressive soils against banana soil-borne diseases, thereby raising the question, "what is the disease suppression ability of pepper residues applied to conducive and suppressive soils and what are the key responsive physicochemical factors?".

In this study, we used banana and its *Fusarium* wilt disease caused by *Fusarium oxysporum* as a model, collected conducive (high-DI) and suppressive (low-DI) soil from the area of a long-term localization trial of banana plants and designed banana residue and pepper residue addition treatments to determine the physicochemical properties and changes in culturable microbes in conducive and suppressive soil and to verify the effect of plant residue extracts on the pathogen. We aimed to investigate the key soil physicochemical factors and their mechanisms of disease suppression in conducive and suppressive soils. We used suppressive soil as a fine criterion, and only the significantly increased physicochemical values in suppressive soil were identified as key response factors compared to those in conducive soil. We hypothesized that the addition of plant residues to banana conducive and suppressive soils to improve soil physicochemical properties would induce an increase in culturable beneficial microorganisms, thereby inhibiting the growth of pathogens and ultimately reducing banana DI.

## 2. Materials and Methods

### *2.1. Crop Residue Preparation and Design of Greenhouse Pot Trials*

In this study, all banana plant (*Musa acuminata* Cavendish cv. Brazil) and pepper plant (*Capsicum frutescens* L., variety Haijiao-309) residues were collected from the plants at their peak growth stage. These plant residues were collected in February 2016 from a field located at Wanzhong Farm (108.45° E, 18.38° N) in Ledong County, Hainan Province, China. All residues were placed in ice boxes and transported back to the laboratory for the next step of processing. The main operations were as follows [11]: first, all residues were gently washed three times with sterile deionized water, and then the cleaned residues were cut into 3 cm equal pieces using a sterilized kitchen knife and ground down to powder. Finally, all residues were uniformly sieved through a 4 mm mesh, and the total nutrient content was measured [25] (Table 1).

**Table 1.** Nutrient content and correction of pepper and banana residues.

| Residue | Nutrient Content | | | Nutrient Content Corrections | | |
|---|---|---|---|---|---|---|
| | N (g/kg) | $P_2O_5$ (g/kg) | $K_2O$ (g/kg) | $CO(NH_2)_2$ (g/pot) | $Ca(H_2PO_4)_2$ (g/pot) | $K_2SO_4$ (g/pot) |
| CK [a] | 0 | 0 | 0 | 3.81 | 2.52 | 13.61 |
| Banana | 10.50 | 3.35 | 70.75 | 1.52 | 1.23 | 0 |
| Pepper | 17.51 | 6.55 | 35.35 | 0 | 0 | 6.81 |

[a] No crop residue added.

According to our previous study [5], a banana orchard continuously cropped over the last 15 years with a sustained low *Fusarium* wilt DI (30% in 2015) was denoted as the disease-suppressive soil. A colocated orchard with high *Fusarium* wilt DI (60% in 2015), also planted with banana for 15 years, was referred to as the disease-conducive soil. Both the suppressive and conducive soils used in the greenhouse pots were dry red soils, which were classified as loam sandy; the sampling site information is shown in Table 2. The potting trials were conducted in a greenhouse on Wanzhong Farm, Hainan, from March 2016 to June 2016 with a daytime temperature of 25–35 °C. There were six treatments in the experiment: (1) C_CK, conducive soil without added residue (control); (2) C_BR, conducive soil with 2% (*w/w*, dry soil weight) banana residue; (3) C_PR, conducive soil with 2% (*w/w*, dry soil weight) pepper residue; (4) S_CK, suppressive soil without added residue (control); (5) S_BR, suppressive soil with 2% (*w/w*, dry soil weight) banana residue; and (6) S_PR, suppressive soil with 2% (*w/w*, dry soil weight) pepper residue. The trial was carried out using a complete randomized block design, and each treatment was replicated three times, with 10 pots (30 cm × 20 cm, diameter × height) per replicate. Each pot was planted with only one banana-tissue-cultured seedling (*Musa acuminata* Cavendish cv. Brazil.). Each pot contained 5 kg of banana soil. Pepper and banana fresh residue (100 g/pot) was added at a ratio of 2% (*w/w*) with respect to soil weight. This amount is consistent with the incorporation of residues in the field settings [26]; the soil and plant residues were then mixed thoroughly and left to decompose in the greenhouse. Tap water (500 mL) was added to each pot if necessary. Each individual treatment was adjusted to equalize the amounts of total nitrogen (TN), total phosphorus (TP) and total potassium (TK) based on the nutrient availability determined for each residue. Adjustments of TN, TP and TK levels were carried out using mineral fertilizers (Table 1). The disease incidence (DI) and biomass of banana plants were counted after 90 days.

**Table 2.** Basic information on the soil for experimental background.

| Target | Conducive Soil | Suppressive Soil |
|---|---|---|
| Site | Jianfeng Town | Jianfeng Town |
| Latitude | 18.753° N | 18.723° N |
| Longitude | 108.648° E | 108.688° E |
| Temperature (°C) [a] | 24 | 24 |
| Precipitation (mm) [b] | 1150 | 1150 |
| Monocultured year | 15 | 15 |
| Soil type | Dry red | Dry red |

[a] The annual mean temperature in 2015. [b] The annual mean precipitation in 2015.

### *2.2. Disease Incidence Determination*

Once the typical symptoms of banana *Fusarium* wilt disease appeared in the pots, we started to monitor the plants weekly for classic symptoms [27], e.g., the vascular tissue becomes dark brown to black, leaves show chlorosis and wilting and pseudostems split longitudinally above the soil. The number of infected banana plants was recorded after the emergence of symptoms had largely stabilized. Disease incidence was calculated as the percentage of diseased plants among the total plants.

### *2.3. Banana Biomass Determination*

In this study, only banana plants without obvious infection symptoms were sampled for biomass determination. After the roots were washed using tap water to remove adhered soils, the plants were brought back to the laboratory on ice and placed in envelopes. Then, the plants were weighed in envelopes, subjected to high-temperature desiccation at 105 °C for two hours and further dried at 75 °C to a constant weight. The final dry weight is the biomass.

### *2.4. Collection of Soil Samples*

In this study, soil samples were collected from healthy banana plant pots. A small shovel was used to collect soil from the 0–10 cm layer in each pot. Three pots were used as one mixed sample. Three points were collected from each pot, and the final nine collected sample points were mixed into one sample. Each soil sample was mixed, homogenized and sieved through a 2 mm nylon sieve to remove plant debris, after which it was divided equally into two subsamples. One subsample was air-dried for physicochemical analysis, and the other was stored at 4 °C for microbiological analysis.

### *2.5. Determination of Soil Chemical Properties*

Soil basic chemical properties were determined according to previous methods [24]. Soil pH was determined with a portable pH meter (Mod. 150, IQ Scientific Instruments, San Diego, CA, USA) at a soil-to-water ratio of 1:5 (*w/v*). Soil organic matter (OM) was determined using the potassium dichromate external heating method [28]. Soil alkali-hydrolyzable nitrogen (AN) was measured by the alkaline hydrolysis diffusion method [25]. Soil available phosphorus (AP) was extracted with sodium bicarbonate, and available potassium (AK) was extracted with ammonium acetate; the corresponding concentrations were determined using the molybdenum blue method and flame photometry, respectively.

### *2.6. Determination of Soil Culturable Microbes*

The number of culturable microbes in the soils was determined by the standard 10-fold dilution plating method. The amount of culturable *Fusarium* was measured using Komada's medium ($K_2HPO_4$ 1 g, $MgSO_4$-$7H_2O$ 0.5 g, KCl 0.5 g, Fe-Na-EDTA 0.01 g, L-asparagine 2 g, galactose 10 g, agar 16 g, deionized water fixed to 900 mL, autoclaved and cooled to 60 °C, 100 mL of salt solution (pentachloronitrobenzene 75% wettable powder 0.9 g, Oxgall 0.45 g, $Na_2B_4O_7$-$10H_2O$ 0.5 g, streptomycin sulfate 0.3 g and pH adjusted to 3.8 ± 0.2 with 10% phosphoric acid [29])). The plates were incubated at 28 °C for 96 h. The amount of

culturable *Pseudomonas* was counted using CFC selective medium (gelatin peptone 16 g, acid hydrolyzed casein 10 g, potassium sulfate 10 g, magnesium chloride 1.4 g, glycerol 10 g, agar 12 g, pH 6.9–7.3, deionized water 1000 mL, autoclaved at 121 °C for 15 min. Before pouring the plate, one unit of *Pseudomonas* CFC selective medium additive was added per 200 mL of medium when the medium temperature dropped to approximately 55 °C), and incubation was performed at 30 °C for 120 h for counting. The amount of culturable *Bacillus* was determined by using Luria–Bertani (LB) medium (peptone 10 g, yeast powder 5 g, sodium chloride 10 g, deionized water 1000 mL, agar 2–2.5 g per 100 mL, 1000 mL of deionized water, pH 7.2–7.4, autoclaved at 121 °C for 20 min) and incubated at 37 °C for 36 h for counting, but the dilutions were heated in an 85 °C water bath for 15 min before coating. The number of colonies growing on the culture count plate was converted into the number of colonies formed per gram of dry soil (colony-forming unit, CFU) and expressed as log CFU $\times$ $g^{-1}$ (dry soil).

A total of 10 g of soil was put in a 150 mL triangular flask containing 90 mL of sterile water. A total of 3–5 sterile glass beads were added, and the flask was placed on a shaker at 170 r/min for 30 min and allowed to stand for 5 min. Then, a pipette was used to aspirate 100 μL of soil suspension into a 1.5 mL sterile centrifuge tube containing 900 μL of sterile water, which was mixed thoroughly with a vortex; this suspension corresponded to a dilution of $10^{-1}$. The above soil suspensions were diluted to $10^{-2}$, $10^{-3}$, $10^{-4}$ and $10^{-5}$ in this order. Using a pipette, 100 μL of soil suspensions at different dilutions was aspirated onto each medium, and each dilution was repeated three times. The dilutions corresponding to culturable *Fusarium* counts were $10^{-1}$ and $10^{-2}$; those corresponding to culturable *Bacillus* were $10^{-4}$ and $10^{-5}$; and those corresponding to culturable *Pseudomonas* were $10^{-4}$ and $10^{-5}$.

### *2.7. Preparation of Plant Residual Extracts*

Plant residue extracts were obtained by the distilled water immersion method. Intact pepper and banana plants were washed, subjected to high-temperature desiccation at 105 °C for 30 min and dried at 80 °C to a constant weight, and the dried samples were crushed and sieved. First, 30 g of the crushed and sieved residue was placed in a triangular flask with 300 mL of sterile deionized water and then extracted at room temperature for 24 h on a 170 r/min shaker. After that, the extract was filtered through a sterile 0.22 μm-pore membrane with a syringe and then stored in a −20 °C freezer.

### *2.8. Determination of the Inhibitory Effect of Plant Residue Extracts on the Growth of Pathogens*

Sterilized 1/2-strength PDA medium was cooled to approximately 55 °C. Then, 2 mL of the corresponding concentrations of plant residue extract and 18 mL of culture medium were accurately pipetted into a sterile plate, shaken well and placed in a workbench for 30 min to sterilize and blow-dry, with the addition of an equal volume of sterile water instead of extract as a control. The concentration of the residual extract in the culture medium was used as the test concentration, which was 0.1%, 1% and 10%. A total of seven treatments were set up with 10 replicates per treatment. *Fusarium oxysporum* f. sp. *cubense* (FOC) disks were inoculated in the center of PDA plates and incubated in an inverted position in an incubator at 28 °C, and the mycelial diameter size of the pathogens was measured after 120 h.

### *2.9. Statistical Analyses*

All statistical analyses were performed using the IBM SPSS 20.0 software program (IBM Corporation, New York, NY, USA) and R software programs (Version 4.2.0). All statistical tests performed in this study were considered significant at $p < 0.05$. To determine significant differences, Tukey's HSD test and one-way ANOVA were performed. The fold change in each test indicator in treatments with crop residue addition (BR and PR) relative to those without crop residue addition (CK) was calculated using the following formula: (B−N)/N, where B is the value of the indicator after adding the residue samples

(BR and PR); and N is the value of the indicator without adding the residue samples (CK) [30]. We used suppressive soil as a fine criterion, and only the significantly increased physicochemical values in suppressive soil compared to those in conducive soil were identified as the key response factors. A piecewise structural equation model (SEM) was developed to explore causal interpretations of how the soil property variables and pathogen abundance affected the disease incidence outcomes. Models were globally fitted using Fisher's C statistic, and coefficients of determination ($R^2$) values and were analyzed with the piecewise SEM package in R [31].

## 3. Results

### *3.1. Changes in the Biomass and Disease Incidence of Banana after Adding Residues to Conducive and Suppressive Soil*

We planted bananas in both naturally conducive and suppressive soils collected after incorporating banana and pepper residue at 2% of the soil dry weight (Figure 1a,b). We found that banana biomass was significantly higher in the suppressive soil than in the conducive soil before any residues were added (left inset of Figure 1c). We also found that banana biomass was significantly higher in suppressive soil than in conducive soil regardless of banana or pepper residue addition (Figure 1c). Meanwhile, the biomass of the treatment with pepper residue was significantly higher than those of the treatment without residue and the treatment with banana residue in both conducive and suppressive soils (Figure 1c). We further calculated the fold change in biomass between the treatments with and without residues and found that the biomass difference was higher in the suppressive soil with pepper and banana residues than in the conducive soil (right inset plot of Figure 1c). We then counted the DI of banana plants and found that the DI of banana was significantly lower in the suppressive soil than in the conducive soil before any residue was added (inset of Figure 1d). Further, we observed that the addition of pepper residues significantly reduced the DI of banana compared with the control (CK) in both conducive and suppressive soils, while DI in the soils treated with banana residues was significantly greater than that in the CK soils (Figure 1d). These results indicate that the suppressive soil mediated by pepper residues would acquire stronger disease suppression ability and thus result in better banana plant growth.

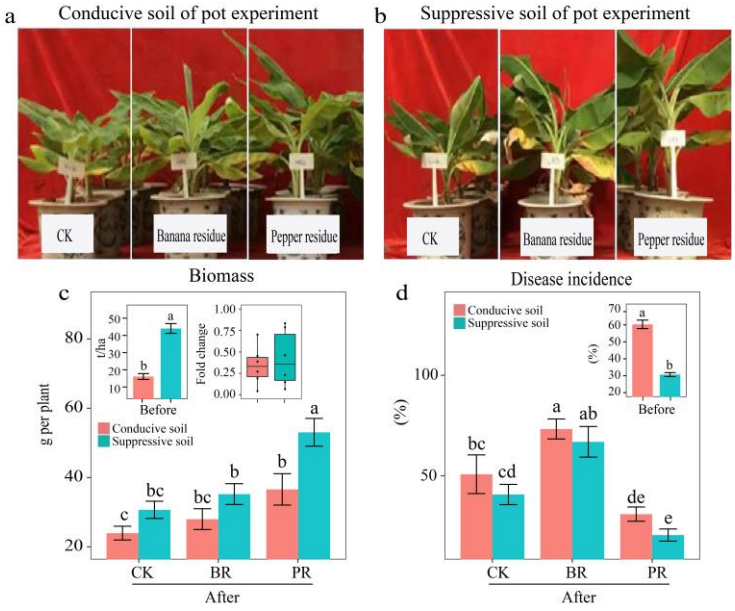


**Figure 1.** Experimental scenarios for conducive soil with different crop residues (**a**); suppressive soil with different crop residues (**b**); changes in biomass of banana plants after additions of different crop

residues to conducive soil and suppressive soil (**c**); the left inset shows the difference in the biomass of the background soil collected before any residues were added, and the right inset shows the fold change in biomass with and without crop residue additions and the changes in disease incidence of banana plants (**d**); inset shows the differences in the disease incidence between the conducive soil and suppressive soil before any residues were added. CK: no crop residue added; BR: banana residue added; PR: pepper residue added. Different lowercase letters indicate statistically significant differences ($p < 0.05$) according to Tukey's HSD test.

### *3.2. Soil Physicochemical, Culturable Microbes and Disease Suppression Effect of Residue Extract*

We found that the pH, alkali-hydrolyzable nitrogen (AN), available phosphorus (AP), organic matter (OM) and available potassium (AK) contents of the suppressive soil were significantly higher than those of the conducive soil before any residue was added (Figure 2a). It was further observed that after the addition of either banana or pepper residues, the nutrient content of the suppressive soil, such as the AN, AP and OM of the soil, was significantly higher than that of the conducive soil compared to the CK, but the pattern of AK was reversed. Meanwhile, we also found that the nutrient content of soil with pepper residue (e.g., AN and AP) was significantly higher than that of soil without residue (CK) and with banana residue in both conducive and suppressive soils (Figure 2a). Further calculations of the fold changes in soil physicochemical differences between the treatments with and without residue addition interestingly revealed that the pH value and AP, OM and AK contents were higher in the conducive soil than in the suppressive soil (significant increase in AK contents), but only the AN content was significantly lower in the conducive soil than in the suppressive soil (inset plot of Figure 2a). This result suggests that AN content is the factor that responds most strongly to residues (especially pepper residues) when applied to conducive and suppressive soils. We speculate that the higher its content in suppressive soils, the stronger the direct or indirect correlation with the disease suppression ability of the soil may be.

In terms of culturable microbes (Figure 2b), we found that the amount of culturable *F. oxysporum* was significantly lower in suppressive soils than in conducive soils before the addition of any residues, while the opposite trend was observed for culturable *Pseudomonas* and *Bacillus*. It was further found that both conducive and suppressive soils had significantly lower amounts of culturable *F. oxysporum* and significantly higher amounts of beneficial culturable *Pseudomonas* and *Bacillus* in pepper-residue-mediated soil compared to CK soil, but the trend was reversed for banana residue. In addition, the amount of culturable *F. oxysporum* was significantly and positively correlated with the DI, while the amount of culturable *Pseudomonas* and *Bacillus* was significantly and negatively correlated with the DI. Moreover, the amount of culturable *Pseudomonas* and *Bacillus* in suppressive soils was significantly and positively correlated with biomass (Figure S1). Further banana and pepper residues were extracted, and we found that the banana residue extract did not significantly inhibit the growth of *F. oxysporum* mycelium compared to the treatment without the addition of residue, but the pepper residue extract did (Figure 2c), and the effect was significantly enhanced with increasing concentration (Figure S2).

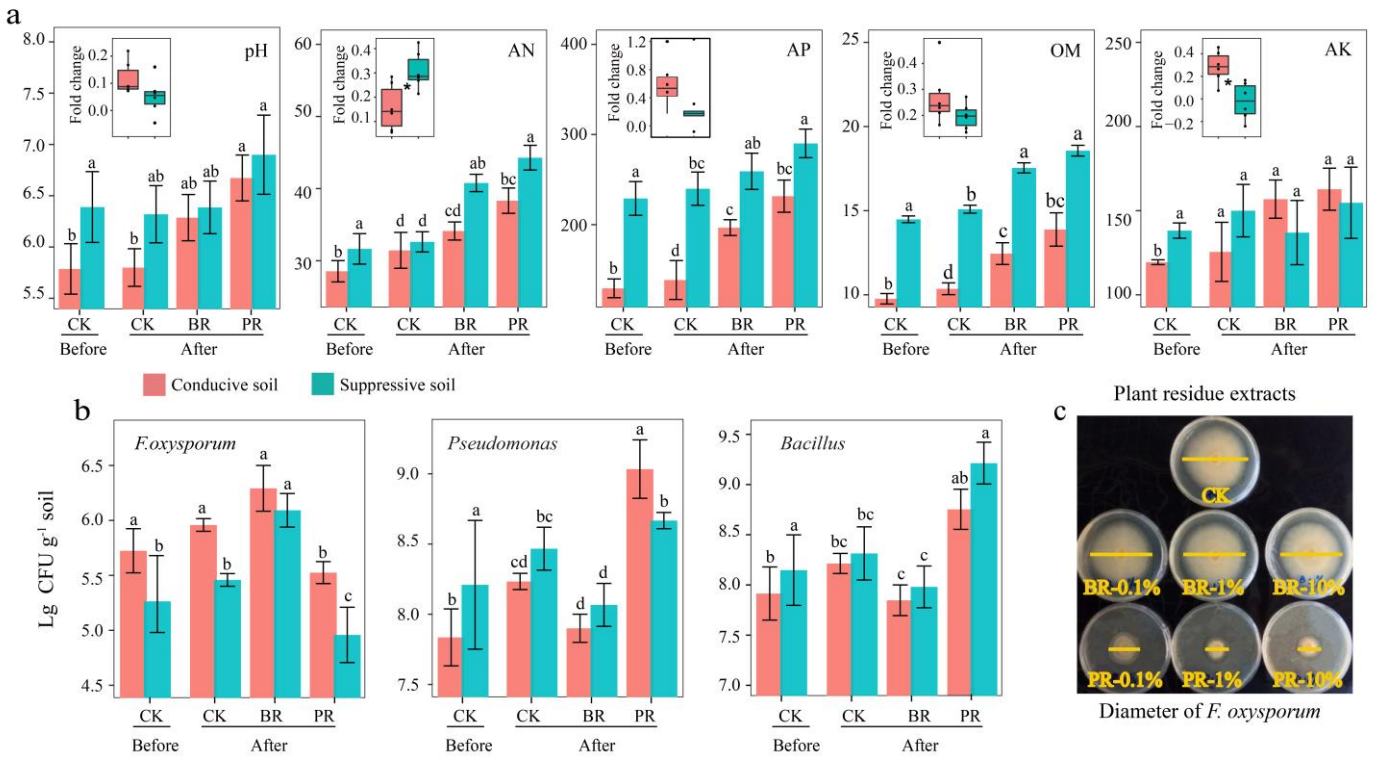


**Figure 2.** Changes in soil physicochemical properties upon the addition of different crop residues to conducive and suppressive soils (**a**) (inset shows the fold change in soil properties between treatments with and without crop residue addition, asterisks indicate significant differences ($p < 0.05$) according to Student's *t*-test), changes in soil culturable microorganisms (**b**) and inhibition of mycelial growth of the fungus *Fusarium oxysporum* f. sp. *cubense* race 4 by different crop residue extracts (**c**). pH: pH value; AN: soil alkali-hydrolyzable nitrogen; AP: soil available phosphorus; OM: soil organic matter; AK: soil available potassium; CK: no crop residue added; BR: banana residue added; PR: pepper residue added; Before: comparison of soil physicochemical properties and culturable microorganisms between conducive and suppressive soil without adding any residues; After: changes in soil physicochemical properties and culturable microorganisms after 90 days of adding residues. Different lowercase letters indicate statistically significant differences ($p < 0.05$) according to Tukey's HSD test (statistically significant differences in the data of samples before the addition of any residue were based on Student's *t*-test).

### *3.3. Effect of the Soil Physicochemical Association of Culturable Microorganisms on Banana Disease Incidence*

To investigate the direct or indirect interactions between soil physicochemical factors, culturable microorganisms, banana biomass and DI, we first directly performed Pearson correlation analysis among soil physicochemical factors, banana biomass and DI. We found that the AN, AP and OM contents of suppressive soil were significantly and positively correlated with banana biomass (Figure 3a). Further, to our surprise, we observed that none of the soil physicochemical factors were directly and significantly correlated with DI in either conducive or suppressive soils (Figure 3b). Accordingly, we speculate that there may be an indirect disease-suppressing relationship between soil physicochemical factors and banana DI. We then constructed a piecewise SEM with soil physicochemical factors as the measured variables and culturable microorganisms and banana DI as the response variables to resolve the potential indirect pathways of disease suppression influenced by soil physicochemical factors in residue-mediated suppressive soils. Interestingly, the piecewise SEM results indicate that although the overall pathway models constructed for pH, AN and AP content of soil physicochemical properties were significant ($p > 0.05$), only the AN factor could indirectly and significantly reduce banana DI by affecting the amount of culturable *Bacillus*

in the bacterial community (Figure 3c). Specifically, first, soil AN significantly increased the amount of culturable *Bacillus* (standardized coefficients = 0.475), then *Bacillus* significantly antagonized the proliferation of *F.oxysporum* (standardized coefficients = −0.789), and finally banana DI was declined by the reduction in the number of pathogens in the soil (standardized coefficients = 0.933).

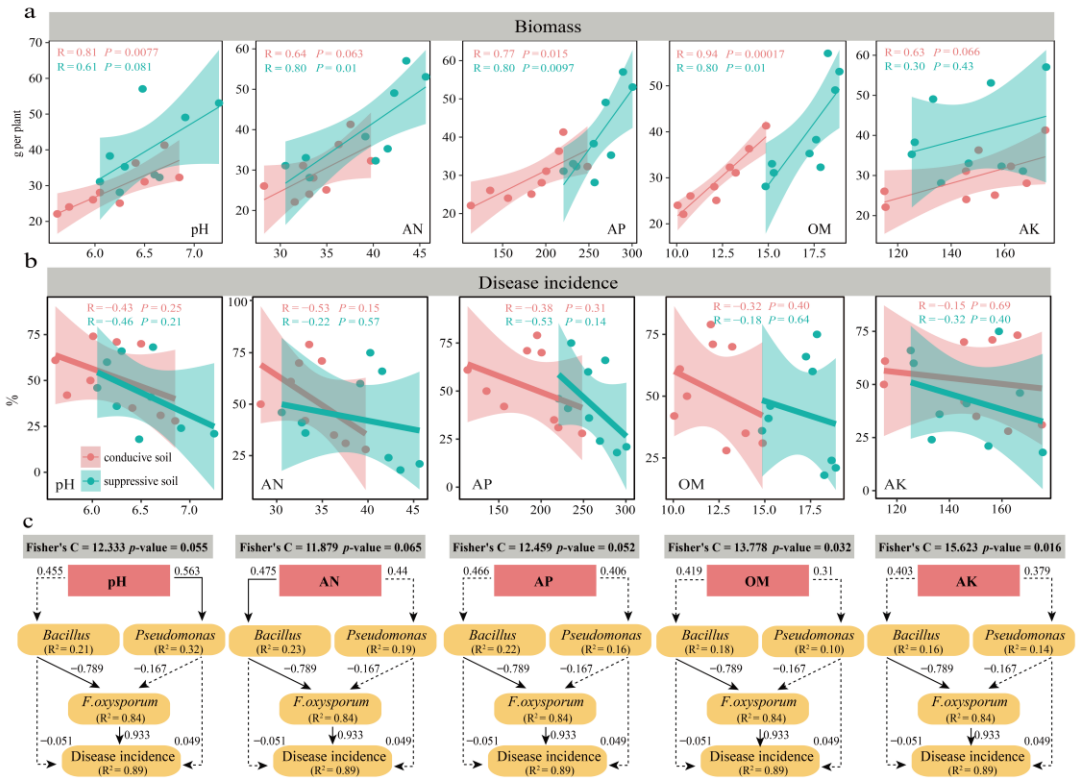


**Figure 3.** Pearson correlations between soil physicochemical factors and banana plant biomass (**a**) and DI (**b**). Piecewise structural equation model (SEM) showing the direct and indirect analysis of soil physicochemical factors determining disease suppression in conducive and suppressive soils after adding different crop residues (**c**). Measured variables are represented by the red boxes, and coefficients of determination ($R^2$) values are provided for response variables by the yellow boxes. Solid arrows represent significant correlation paths with $p$ values < 0.05, dashed arrows represent non-significant paths, and the value next to the path represents the path coefficients (standardized coefficients). A positive value of the path coefficient represents a positive relationship; otherwise, it represents a negative relationship.

## 4. Discussion

Numerous studies have shown that disease-suppressive soils for various plant diseases are effective against invasion by soil-borne pathogens [5,7,17]. Crop residues returned to the field can also induce conducive soils to form suppressive soils and thus acquire disease-suppressing ability, the exertion of which is influenced by the synergy of biotic and abiotic factors [6,32]. However, the residue-mediated differences in the disease suppression ability of conducive and suppressive soils have not yet been reported from a soil physicochemical perspective. In this study, we used *Fusarium* wilt of banana as a model to investigate the physicochemical properties and changes in culturable microorganisms and verify the disease suppression effect of residue extracts by adding banana and pepper residues to the conducive and suppressive soils.

The results of the changes in biomass and DI difference between our residue-added and non-added treatments indicate that the pepper-residue-mediated suppressive soil significantly increased the biomass and effectively suppressed the occurrence of banana

*Fusarium* wilt. Further results demonstrate that the disease suppression ability of the residue-mediated suppressive soil was significantly correlated with the species (crop type) of the residue. These findings are consistent with the results of previous studies. For example, mustards, rapeseed and sudangrass were returned to the field as residue green manure, and all significantly reduced potato DI and increased potato yield, but mustards were indeed the green manure crop variety that was the most effective in suppressing soil-borne diseases of potato [33]. Additionally, cover crop variety type diversity increased crop yields more than single additions [34]. Taken together, these results suggest that residues can be used as a medium to induce conducive soils to acquire disease-suppressive abilities; in other words, they play a supportive role, and the essential difference that determines disease-suppressive abilities between conducive and suppressive soils may be the difference in soil environmental conditions themselves [35].

Maintaining a balance of nutrient content in the soil plays a key role in the disease suppression capacity of the soil [36], and a high nutrient content in the soil tends to have a higher disease suppression capacity [5]. For example, it has been shown that soil organic nitrogen content is negatively correlated with *Fusarium* wilt [37]. Similarly, our study found that the fold change in soil AN was significantly higher in suppressive soil with and without residue addition treatment than in conducive soil, and the addition of pepper residue was also more effective than the addition of banana residue and without residue (CK). The above results indicate that the soil AN factor responded most strongly to pepper residues after application to both conducive and suppressive types of soil. Accordingly, we hypothesized that the increased nitrogen content in the residue-mediated suppressive soil increased the biomass of banana, and the banana plant grew better and was less susceptible to invasion by pathogens, thus enhancing the disease resistance of the banana plant.

In this study, it was found that the amount of culturable *F. oxysporum* and the amount of culturable *Pseudomonas* and *Bacillus* increased significantly in the pepper-residue-mediated suppressive soil. This finding is supported by many studies on soil bacterial community composition and plant disease suppression functions [38], which confirm that many of the probiotic bacteria closely associated with the disease-suppressive capacity of plant suppressive soil are derived from either *Pseudomonas* or *Bacillus* [17,39]. In summary, the most important reason for the residue-mediated improvement in soil disease suppression may be the reduction in the number of culturable pathogens in the soil [40] and the increase in the number of culturable beneficial bacteria in the soil, especially the amount of culturable *Bacillus* in the soil. In other words, in suppressive soils with high pathogen stress, the addition of pepper residues induces effective colonization of *Bacillus* in the banana rhizosphere, thereby antagonistically blocking pathogen invasion and ultimately effectively reducing the DI of banana [41].

To further confirm the disease suppression effect of pepper residues, we extracted the pepper residues. It was found that the pepper residue extracts significantly inhibited the growth of the pathogen mycelium, and the higher the concentration was, the better the inhibition effect. This result is also supported by other studies where residue extracts of pepper were inoculated in a beef paste medium with good inhibition of *Salmonella typhimurium* and *Pseudomonas aeruginosa*, and a significant fungicidal effect occurred with increasing concentrations of the extracts [42]. However, it is worth noting that which specific substance in the pepper extract inhibited the growth of *F. oxysporum* mycelium needs to be deeply explored.

To further clarify the direct or indirect interactions among soil physicochemical factors, soil culturable microorganisms, biomass and DI, we conducted Pearson correlation analysis and constructed piecewise SEM for these factors. We found that AN content was significantly and positively correlated with banana biomass, but not significantly and negatively correlated with DI. Based on this finding, we hypothesized that there might be an indirect correlation with the DI. Indeed, our further analysis revealed that only a significant increase in soil AN content led to an increase in the amount of culturable *Bacillus*, and the increased beneficial *Bacillus* in the soil significantly inhibited the proliferation of *F. oxysporum*, which

was ultimately effective in indirectly reducing the DI of banana (SEM analysis). Recent studies have reported a phenomenon similar to ours in that soil physicochemical factors were the largest explanatory factors affecting changes in bacterial richness and community composition in 471 soil samples, particularly soil water content (SEM analysis) [43]. However, it is worth noting that although the availability of nitrogen in the soil plays a keystone role in the microbial community by influencing the occurrence of crop diseases [44], the form of nitrogen (nitrate or ammonium) by which pathogen proliferation is regulated needs further research [45]. Additionally, the above findings support our hypothesis that the addition of plant residues to banana conducive and suppressive soils increased the content of soil AN, inducing an increase in the amount of culturable *Bacillus* and thereby suppressing pathogen growth and ultimately showing effectiveness in indirectly reducing banana DI.

## 5. Conclusions

The naturally suppressive soil increased banana biomass and reduced banana DI before any residue was added. The biomass in the suppressive soil was consistently higher than that in the conducive soil due to the incorporation of either banana or pepper residues, but only the soil with pepper residues reduced the DI of banana. Pepper residues applied to both conducive and suppressive soils reduced the amounts of culturable *F. oxysporum* and increased the amounts of beneficial *Pseudomonas* and *Bacillus*. The pepper residue extracts also showed a good inhibitory effect on pathogen. Among all soil physicochemical factors, the soil AN factor responded most strongly to the residues after application to both soils. In addition, the soil AN factor was not directly related to banana DI, but it could stimulate the amount of culturable *Bacillus* in the soil, and the increase in the amounts of culturable *Bacillus* antagonized the proliferation of pathogen, thus indirectly and effectively reducing banana DI. The results of this study may offer novel insights into the difference in disease suppression ability between conducive and suppressive soils from a physicochemical point of view, and also provide a reference for the relevant disease suppression mechanism after green manure return to the field settings.

**Supplementary Materials:** The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agronomy12092077/s1, Figure S1: Pearson correlation between soil culturable microbes and biomass and disease incidence after adding different crop residues to conducive and suppressive soil. Figure S2: Effect of different residue extract concentrations of pepper and banana plant on the diameter of fungus *Fusarium oxysporum* f. sp. *cubense* race 4.

**Author Contributions:** S.H. and Y.R. designed the experiments. S.H. and H.J. performed the experiments. S.H. analyzed the data. S.H. and Z.J. wrote the manuscript. X.Y., J.G., B.W., Y.Z., Q.W. and R.L. gave valuable comments on the manuscript. Y.R. and Z.J. reviewed and edited the manuscript. All authors have read and agreed to the published version of the manuscript.

**Funding:** This research was supported by the National Natural Science Foundation of China (32160750, 31672239), the Project of Sanya Yazhou Bay Science and Technology City (SCKJ-JYRC-2022-94), National Key Basic Research Program of China (2015CB150503), Central Public-interest Scientific Institution Basal Research Fund (NO. 1630142022005) and the Innovation Platform for Academicians of Hainan Province.

**Institutional Review Board Statement:** Not applicable.

**Informed Consent Statement:** Not applicable.

**Data Availability Statement:** Not applicable.

**Conflicts of Interest:** The authors declare no conflict of interest.

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